



Animal and Plant  
Health Inspection  
Service

Veterinary  
Services

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# H5/H7 Avian Influenza

## Case Definition

- 1. General disease/pathogen information:** Avian influenza (AI) is a viral infection of birds including chickens, turkeys, guinea fowl, and other avian species. Certain subtypes of AI can cause zoonotic disease, with most human cases occurring after direct contact with infected poultry. Wild waterfowl and shore birds are considered the natural reservoir for AI viruses. The severity of disease may range from inapparent infection to reproductive disturbance (loss of egg production), respiratory disease, or an acute and fatal systemic disease. Morbidity and mortality rates generally depend on the pathogenicity type of the virus involved. There are three general categories of AI viruses in birds: low pathogenicity avian influenza (LPAI) non-H5/H7 viruses, LPAI H5/H7 viruses, and high pathogenicity (HPAI) viruses. The non-H5/H7 viruses typically do not cause disease in poultry although swine lineage viruses (H1 and H3) may cause reduced fertility and performance in turkeys; LPAI H5/H7 is usually mild and may cause low morbidity and mortality in poultry; and HPAI<sup>1</sup> typically causes high morbidity and mortality. AI reportable to the World Organization for Animal Health (OIE) are those infections of poultry caused by any influenza A virus of the H5 or H7 subtype or other subtypes meeting specific requirements for high virulence (i.e., severity of disease defined by pathogenicity index or amino acid sequence in the hemagglutinin receptor protein).
  - 1.1. Etiologic agent:** The agent responsible for avian influenza is an orthomyxovirus. Influenza viruses are classified by examining nuclear and matrix proteins that divide them into three groups: influenza types A, B, and C. All influenza viruses from birds and most from mammals are type A. Type A influenza viruses are further classified into subtypes based on antigenic differences of two surface proteins, hemagglutinin (HA) and neuraminidase (NA). Currently, 16 HA and 9 NA subtypes are broadly recognized.
  - 1.2. Distribution:** AI occurs worldwide. HPAI outbreaks have recently occurred in Southeast Asia and Europe. AI viruses tend to circulate within migratory bird populations that follow specific flyways (e.g., H7 viruses found in wild bird populations in the Americas are more closely related to each other compared to H7 viruses found in wild birds in Europe or Asia). The Asian lineage H5N8 HPAI clade 2.3.4.4 virus spread rapidly along wild bird migratory pathways in the Eastern Hemisphere during 2014 (Lee, et al. 2015). Introduction of this virus into the Pacific Flyway of North America sometime during 2014 allowed mixing with North American (AM) origin low pathogenicity avian influenza A viruses generating new (novel) combinations with genes from both EA and AM lineages (so-called “reassortant” H5Nx HPAI viruses). Other outbreaks of HPAI in the United States occurred in 1924, 1983, and 2004. Incidents of non-H5/H7 LPAI subtypes of low concern are commonly detected in U.S. domestic poultry flocks and live bird markets.
  - 1.3. Clinical signs:** AI virus infections in domestic poultry may be clinically inapparent or result in disease that ranges from mild transient clinical signs to 100 percent morbidity and/or mortality, depending on virus pathogenicity type (Swayne and Suarez 2000). In addition to pathogenicity,

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<sup>1</sup> Currently only H5/H7 subtypes recognized as highly pathogenic

other factors such as genetics, nutrition, and co-infection with other pathogens affect clinical outcome. When seen, clinical signs may be evident as respiratory, enteric, cardiovascular, or reproductive.

- 1.3.1. LPAI H5/H7:** Often cause either no disease signs or result in mild cases, but may cause increased mortality, decreased feed consumption, respiratory signs (e.g., nasal discharge, coughing, sneezing), and decreased egg production (Dunn *et al.* 2003).
- 1.3.2. HPAI:** Have a greater level of illness and could exhibit one or more of the aforementioned clinical signs and any of the following clinical signs: sudden death, lack of energy and appetite, soft-shelled or misshapen eggs, swelling and purple discoloration of the combs or wattles, hemorrhages on the unfeathered parts of legs and feet, lack of coordination, and diarrhea (Elbers *et al.* 2005).
- 1.4. Incubation period:** The incubation period is dependent upon the AI virus strain, dose, route of exposure, and species of bird. The incubation period can range from 1-7 days. OIE recognizes a 21-day incubation period for virus spread in a bird population.
- 1.5. Differential diagnosis:**
  - 1.5.1. Viral diseases:** Newcastle disease or other paramyxoviruses, infectious laryngotracheitis, infectious bronchitis, avian metapneumovirus in turkeys
  - 1.5.2. Bacterial diseases:** fowl cholera, mycoplasmosis, infectious coryza, ornithobacteriosis
  - 1.5.3. Fungal diseases:** aspergillosis
- 1.6. Transmission and reservoir:** AI is spread by direct contact between healthy and infected birds and indirect contact with contaminated equipment and materials. The virus is excreted through the feces of infected birds and secretions from the upper respiratory tract and eyes, and may be dependent upon the course of infection as well as the virus itself. Waterfowl and shorebirds are considered natural reservoirs of influenza A and LPAI H5/H7 viruses. Wild waterfowl generally show no signs of illness due to AI virus infection, may excrete virus in feces for long periods, may be simultaneously infected with multiple subtypes, and often do not develop detectable levels of antibody. Seasonal infection with AI virus occurs in conjunction with hatching, brooding, and fledging of susceptible juveniles (Halvorson D.A. 2002). Influenza A viruses generally remain in evolutionary stasis within wild birds and do not cause mortality (Webster *et al.* 2006).
- 1.7. Epidemiology:** HPAI viruses generally originate in domestic poultry like chickens and turkeys, and usually result in high morbidity and mortality, near 100 percent in infected populations. Certain subtypes of AI are of particular concern for zoonotic transmission with the potential to infect humans and other mammals.

**2. Laboratory criteria:** Subclinical infections identified through active laboratory surveillance or clinical cases with compatible clinical signs and pathologic lesions in a susceptible species are evaluated using laboratory criteria for HPAI and LPAI H5/H7 defined by one or more of the following diagnostic strategies:

- 2.1. Serologic tests:** demonstration of influenza antibody by:
  - 2.1.1.** Agar gel immunodiffusion (AGID) OR USDA-licensed influenza A enzyme-linked immunosorbent assay (ELISA); AND
  - 2.1.2.** Confirmation of antibody to H5 or H7 by hemagglutination inhibition (HI).
- 2.2. Antigen tests:** detect presence of influenza A virus by:
  - 2.2.1.** Antigen capture immunoassays (ACIA): collect tracheal/oropharyngeal and/or cloacal swab samples from clinically ill or dead birds. ACIA (test kits approved by APHIS) are for flock level testing; the ability to detect low levels of infection is enhanced by testing multiple samples. Molecular confirmation of positive results is required; negative results with clinical signs require confirmatory diagnostics as indicated in VS Guidance 12001,

“Policy for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI).” Samples will be forwarded to USDA’s National Veterinary Services Laboratories (NVSL) to determine subtype and pathotype.

- 2.2.2.** Direct RNA detection: Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) using NVSL-approved molecular assays for influenza A and H5/H7 subtypes, WITH molecular determination of subtype and pathotype direct from swab sample by Sanger sequence methods, OR virus isolation with antigenic and/or molecular characterization.
- 2.3.** *Virus isolation and identification:* preferred specimens for virus isolation include tracheal/oropharyngeal and cloacal swabs, or fresh feces from live or dead birds, or samples from organs pooled by system (e.g., respiratory-trachea, lungs, air sacs; enteric-intestine, spleen, kidney, liver; reproductive) from dead birds. A preparation of the specimen is inoculated into the allantoic cavity of susceptible embryonated chicken eggs. The eggs are incubated at 37°C for 4 to 5 days. The amniotic-allantoic fluid is harvested from inoculated embryos and tested for presence of virus by molecular, hemagglutination, or antigen capture methods with subtype (HA and NA) determination by molecular or hemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays.
- 2.4.** *Strain virulence evaluation:*
- 2.4.1.** Determination of the amino acid sequence at the hemagglutinin cleavage site (of H5 and H7 viruses) to identify viruses that have the capacity to become highly pathogenic with or without elevated mortality in *in vivo* assays (see 2.4.2).
- 2.4.2.** Viruses with an intravenous pathogenicity index (IVPI) greater than 1.2, or that cause at least 75 percent mortality within 10 days in 4- to 8-week-old chickens infected intravenously, are classified as HPAI.
- 2.4.3.** If H5 or H7 subtypes do not meet the criteria for HPAI, they are classified as H5/H7 LPAI.
- 2.5.** *Assumptions:* Influenza virus may be detected 48 hours post-infection (HPAI within 24 hours post-infection) by virus isolation or rRT-PCR (Spackman 2006) and 1-5 days post-infection by antigen capture enzyme immunoassay, when virus is shed at moderate to high levels (Gelb and Ladman 2006). Oropharyngeal/tracheal specimens are preferred for poultry because there generally are fewer inhibitors and therefore higher test sensitivity especially during the early phase of infection. While oropharyngeal/tracheal swabs are preferred for detection of AI in poultry, cloacal swabs are more preferred in wild birds. Presence of blood or fecal material in swab specimens (i.e., cloacal swabs) can result in lower sensitivity on the rRT-PCR assay due to the presence of non-specific inhibitors, and should be processed appropriately.

### 3. Case definition:

- 3.1.** *General comments:* AI virus can infect almost all species of birds. Domestic poultry defined as having illness compatible with OIE reportable AI infection (H5/H7 HPAI and LPAI) are those with one or more of the following clinical signs and gross lesions: reduction in normal vocalization; listlessness; conjunctivitis; drops in egg production sometimes with pale, misshapen or thin-shelled eggs; respiratory signs such as rales, snicking, and dyspnea; neurological signs such as incoordination or torticollis; a drop in feed and/or water consumption; swollen or necrotic combs and wattles; swollen head and legs; lungs filled with fluid and blood; tracheitis and airsacculitis; hemorrhages on the unfeathered parts of legs and feet; petechial hemorrhages on internal organs (Easterday *et al.* 1997); OR flocks that experience mortality as listed for each compartment as follows (S. Malladi and E. Gingerich, personal communications, 2013):

- Commercial broilers: mortality exceeding 3.5 birds/1,000 per day

- Commercial layers: mortality exceeding 3 times the normal daily mortality per day (normal: 0.13 birds/1,000 per day for layers from 2 to 50 weeks, and 0.43 birds/1,000 per day for layers over 50 weeks); OR 5 percent drop in egg production for 3 consecutive days
  - Commercial turkeys: mortality exceeding 2 birds/1,000 per day
  - Broiler breeders: mortality exceeding 2 birds/1,000 per day
  - Layer breeders: mortality exceeding 3 times the normal daily mortality per day (normal: 0.2 birds/1,000 per day prior up to 50 weeks, and 0.37 birds/1,000 per day after 50 weeks)
  - Turkey breeders: mortality exceeding 2 birds/1,000 per day; OR a decrease in egg production of 15 percent occurring over a 2-day period
  - Small-volume high-value commercial poultry and backyard flocks: any sudden and significant mortality event or sudden drop in egg production should be investigated
- 3.2. *Suspect case:*** Domestic poultry with:
- 3.2.1.** Illness compatible with H5/H7 AI infection; **OR**
  - 3.2.2.** Positive AGID or ELISA samples taken during routine surveillance with or without the presence of compatible illness; **OR**
  - 3.2.3.** Detection by ACIA using a commercially available influenza A antigen (test kit approved by APHIS) with the presence of compatible illness.
- 3.3. *Presumptive positive case:***
- 3.3.1.** A suspect positive case as defined above with detection of antibodies to influenza A in sera as determined by AGID serological test that cannot be explained by vaccination (USDA permission required for use in the U.S.), and subsequent subtyping by HI and NI as H5 or H7 with any NA subtype; **OR**
  - 3.3.2.** Domestic poultry with identification of influenza A RNA by rRT-PCR with or without the presence of compatible illness.
- 3.4. *Confirmed positive case:*** Domestic poultry with antigen detection (virologic or molecular detection methods) **AND** the confirmation of the H5 or H7 subtype **WITH** subsequent determination of *pathogenicity* as described in Section 2.2 of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (HPAI or H5/H7 LPAI) by NVSL.
- 3.5. *Epidemiological criteria and restrictions:*** Surveillance efforts are restricted along the lines of the compartmentalization concept. Compartmentalization is intended to create a functional separation of the commercial poultry industry, the Live-Bird Marketing System (LBMS), backyard poultry flocks, and wild migratory waterfowl through management practices (Scott 2006). The efficacy of compartmentalization can be verified through surveillance information and evaluation.
- 3.5.1.** Commercial poultry breeder and production flock surveillance (including many game bird breeders) is conducted through the National Poultry Improvement Plan (NPIP).
  - 3.5.2.** Commercial meat-type chicken and meat-type turkey surveillance is an industry initiative of the National Chicken Council and National Turkey Federation that meets or exceeds the NPIP commercial surveillance program.
  - 3.5.3.** LBMS surveillance occurs through cooperative agreements between APHIS and the participating State Animal Health Official (SAHO). The federally funded and State-administered program is designed to enhance and unify existing State programs and to assist States in meeting their goals for prevention and control of H5/H7 LPAI in the LBMS. State programs often exceed APHIS minimum standards.
  - 3.5.4.** Surveillance of the non-traditional backyard compartment occurs through individual State surveillance programs in cooperation with APHIS.

- 4. Reporting criteria:** Suspected cases of HPAI in domestic poultry should be reported in accordance with VS Guidance 12001, "Policy for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI)." Suspected cases or laboratory cases consistent with H5/H7 LPAI in domestic poultry should be reported in accordance with VS Guidance 8602.1 "Response, Communications, and Investigation of Notifiable Avian Influenza (NAI) in Domestic Poultry" and VS Guidance 8604.1, "Reporting Confirmed Findings of Low Pathogenic Notifiable Avian Influenza (LPNAI) (H5 and H7 Subtypes) to the World Organization for Animal Health (OIE) and to Trading Partners." In addition, the SAHO should report the presence or absence of H5/H7 AI in commercial poultry to APHIS through the National Animal Health Reporting System (NAHRS) following NAHRS reporting guidelines.
- 5. Control and surveillance procedures:** There is no current treatment for AI. In the event of an outbreak, the methods of control include immediate depopulation of all birds in the flock, controlled marketing for LPAI H5/H7, disposal of carcasses and all animal products that are not controlled-marketed, and strict biosecurity measures, including a hold of 21 days before restocking the poultry population. Preventative measures enforce surveillance and good biosecurity. Surveillance measures in domestic poultry include observational surveillance, active serological surveillance (large-volume commercial poultry, LBMS), and active antigen surveillance (large-volume commercial poultry, LBMS). Surveillance is also conducted in migratory waterfowl and zoo/exhibition bird populations.

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